CYCLOTELLA CASPIA (BACILLARIOPHYCEAE) IN SOME RIVERS AND LAKES IN EUROPE (MORPHOLOGICAL OBSERVATIONS).

K.T. KISS*, M. COSTE**, R. LE COHU*** and M. NAUSCH****

ABSTRACT. – Electron microscopic investigations showed that *Cyclotella caspia* Grun., known primarily from seas and brackish waters is also to be found in several European rivers and lakes. The species exhibits a great variability of morphological features, mostly based on the degree of silicification of the frustules. Thus, forms with short and long costae, with more circular or longitudinally elongated alveoli could be distinguished. In addition a range of transitional forms could be recognized. The average number of costae measured in 10µm, is smaller than what is written in literature. This difference is probably to be attributed to the use of internationally recommended system (Anonymous, 1975), while the authors used Genkal's method (1977) more suitable for the small species of the Thalassiosiraceae. Our own observations and other literature data show that *Cyclotella caspia* is of general occurrence in the freshwaters of Europe and probably of world wide distribution.

RÉSUMÉ. – Des études en microscopie électronique ont montré que *Cyclotella caspia* Grun., connu à l'origine seulement en milieu marin et saumâtre, se rencontre également dans plusieurs rivières et lacs d'Europe. Cette espèce présente une grande variabilité morphologique liée le plus souvent au degré de silicification des frustules. Côtes longues ou courtes, alvéoles circulaires ou allongées permettent de distinguer différents types avec toute une série de formes de transition. Le nombre moyen de côtes en 10 µm est inférieur à celui donné par la littérature. Cette différence est due au fait que les auteurs utilisent la méthode de Genkal (1977), plus fiable pour les petites espèces de Thalassiosiraceae, et non celle préconisée (Anonyme, 1975). Nos propres observations et d'autres données de la littérature montrent que *Cyclotella caspia* a une large répartition dans les eaux douces non seulement en Europe mais aussi dans d'autres continents.

KEY WORDS : Bacillariophyceae, Centrales, *Cyclotella*, morphology, taxonomy, geographical distribution, Europe.

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INTRODUCTION

The increasingly application of electron microscopy (EM) during the past decades has contributed much new information to our knowledge of the species of the Thalassiosiraceae family. These results are important not only from the aspect of taxonomy, helping to establish the exact taxonomic position of many species, but also of their distribution and ecology.

Several species primarily known from brackish waters and the sea, also occur in freshwaters, and often in appreciable numbers; one such species is *Cyclotella caspia*, which was described by Grunow (1878) from the Caspian Sea. Later, Hasle (1962), Proshkina-Lavrenko (1959, 1963), Sullivan (1978, 1981), Nesterova (1985) and Genkal & Makarova (1986) also encountered it in marine samples. The first information on its freshwater occurrence was by Florin (1970), and this was followed by Foged (1980), Johanssen *et al.* (1983), Archibald (1983) and Nagumo & Kobayasi (1985). On the basis of the electron microscopic material it is confirmed that *C. caspia* also exists in several European freshwater habitats. This paper attempts to give a more complete picture of the morphological features of this species by means of TEM and SEM studies. Information will also be given concerning distribution, quantitative relationship and some ecological aspects of *C. caspia*.

MATERIAL AND METHODS

Samples were collected at the following places (Fig. 1) :

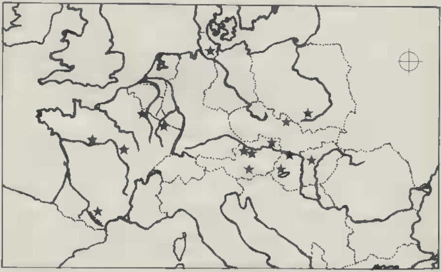


Fig. 1 – Sampling points in the course of the investigation of *Cyclotella caspia*.

Austria - rivers : the Danube (near Klosterneuburg), Traun, Enns (before it meets the Danube); Saisser.

Czechoslovakia - lake : In a small lake near Karolinka village.

France - rivers : the Loire (near Blois), the Rhône (near Avignon), the Meuse and the Moselle (near the Belgium border), Canal du Midi (near Toulouse).

German Federal Republik - river : the Elbe (near Hambourg).

Hungary - rivers : the Danube (near Göd, Budapest) the left bank of Danube (near Cikolasziget), Tisza (near Tokaj, Tiszalök), Szamos (near Vasarosnamény); canals : Eastern Main Canal (near Tiszalök, Balmazujvaros); lake : Lake Balaton (Keszthely bay).

Poland - river : the Vistula (downstream from Goczalkowice Reservoir).

Scooped samples were taken from the near the surface layer and fixed with lugol solution. In some samples, the quantity of phytoplankton was also determined by Utermöhl's method (Lund *et al.*, 1958).

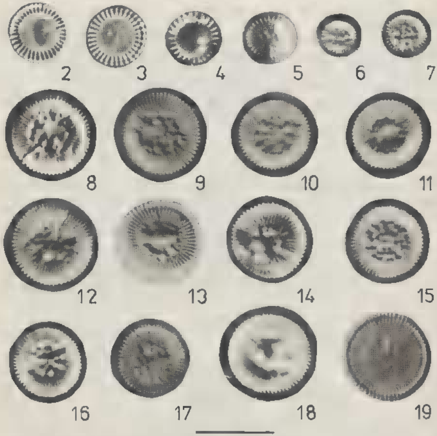
The morphological examinations of *C. caspia* were performed under light and electron microscope. Sedimented plankton samples were digested with hydrogen peroxide and washed with distilled water. For light microscopic examination the cleaned samples were mounted in Naphrax resin (R.I. = 1,74). The usual methods were used for E.M. studies. On the one hand, an OPTON inverted microscope, a ZEISS Amplival microscope, a Nacher NS400 and a Reichert Biovar were used for light microscope studies, a TESLA BS 500, a ZEISS EM 9-S2 and a JEOL 100 S, plus JEOL JSM 35 scanning electron microscope for TEM and SEM studies on the other hand.

The structural elements of the valves were measured and analyzed on the basis of the papers by Genkal (1977), Genkal & Kuzmin (1979a) and terminology after Anonymous (1975) and Ross *et al.* (1979).

RESULTS

1) Morphological features of *Cyclotella caspia* Grunow.

The cells are tiny, with diameters less than 21 µm and rectangular in girdle view. Under the light microscope, only the structure of cells larger than 8-10 µm is discernible. On the valve mantle, densely located costae can be seen, surrounding a structureless central part, which is occasionally covered with minute spots. In the central zone tangential folds are frequent and one stigma can be found (Fig. 2-19). Further details of the structural characteristics of this species have been obtained with the electron microscope. The costae start from the valve mantle and reach about 1/3 of the radius. The number of costae ranges from 9 to 25 in 10 µm (Table 1). The costae are generally short, occasionally longer, with a few dichotoming branchings on the valve mantle. The central part of the valve face has a very characteristic pattern, which is visible mainly in SEM micrographs (Figs. 20-23, 41). The central part with a light tangential fold across it, is superficially ornamented with tiny, irregularly protuberances.



Figs. 2-19 : LM. Valve view of *Cyclotella caspia*. — 2-4 : Specimens from Loire. 5-19 : Specimens from the Danube. Scale 10 μm.

Table 1 : Data and measurements of the valve structure elements of *Cyclotella caspia*.

	D	Nc	Tnmsp	Nmsp	c / m	(D-Dc)/D
min.-max.	3.8-21.0	9.0-25.0	3.0-20	2.6-5.1	2.9-9.7	0.22-0.64
x	8.115	16.618	9.494	4.005	4.233	0.372
Sx	0.370	0.398	0.497	0.076	0.190	0.011
S	3.371	3.590	3.888	0.595	1.503	0.104
S^2	11.229	12.732	14.872	0.348	2.221	0.011
CV	41.540	21.603	40.960	14.856	35.506	27.956

D: diameter of valve; Nc: number of costae in 10 μm; Tnmsp: total number of marginal strutted processes; Nmsp: number of marginal strutted processes in 10 μm; c/m: number of costae divided by number of marginal processes; (D-Dc)/D: diameter of valve minus diameter of central part of valve divided by diameter of valve; x: mean value; Sx: error of mean value; S: corrected scatter; S^2: variance; CV: variation coefficient. Number of data: 81.

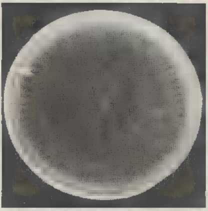
The small circular opening of the central strutted process is of excentric location. On the arched mantle of the valve, the external pores of the marginal strutted processes occur in regular arrangement (Figs. 23-24, 41). In the immediate vicinity of the pores, the costae are somewhat widened. On the valve mantle tiny denticles granules are occasionally present on the costae (Fig. 24). The inner part of the valve is quite different from the outer part. The shape of costae, which are shorter and arched are best visible. The alveoli are generally small, elongated and oval. The tangential fold of the central zone is often more conspicuous on the inner surface than on the outer one (Figs. 25, 99).

The inner tube of each marginal strutted process is about one and half-twice as long as respectively the two satellite pores besides it. Marginal strutted processes generally occur on every third costa, occasionally less frequently. Sometimes they are only visible on every 5-8 costa and they are irregularly distributed (Fig. 27). The central strutted process is more or less excentrically located with three, rarely two satellite pores. In the material examined, only occasionally were observed valves, having two or three central strutted processes (Fig. 28). The external pore of the labiate process is identical with those of the strutted processes. The lips are at times parallel with the costae, sometimes normal to it or obliquely located (Figs. 29, 30). Only a single valve was found, that had two labiate processes (Fig. 28). Sometimes the labiate process does not occur on the valve mantle, but on the valve face at a greater distance from the mantle (Figs. 20, 40).

The structure of the alveolar field between the costate is well seen in the TEM micrographs. In some valves, the alveoli are broadly rounded and relatively short, in other cases more elongated with longitudinal rows of finely perforated pores. In some specimens, more marked siliceous bars develop transversally, forming concentric rings which run parallel with the valve mantle (Figs. 26, 32). In these specimens the pores of the alveoli are circular. Occasionally the costate run a longer distance to the central part, and in such cases the inner rim of alveoli does not appear to be fully closed in the TEM micrographs.

2) Occurrence of *Cyclotella caspia* in a few European freshwaters

Most data about *C. caspia* originate from the Danube and its tributaries. This species was rarely abundant. However, during the vegetation period, the Danube is characterized by a low water level, a low speed of flow and often several ten million phytoplankton individual numbers per liter (Kiss, 1984 a, b, c, 1986). Then the abundance of *C. caspia* occasionally reached 100-150 thousand ind./l in the phytoplankton of the Budapest section of the Danube. It was also twice recorded from periphyton samples. We have very rare data on its occurrence in the Austrian tributaries of the Danube (see Fig. 1). Its presence was noted on 13 occasions in the Tisza, Szamos and the Eastern Main Canal. It was also found on 3 occasions in the Keszthely region of the western basin of Lake Balaton. It also occurs regularly in Lake Saisser in the central part of Austria (7 occurrences).



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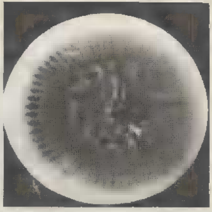

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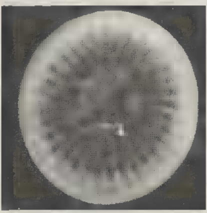

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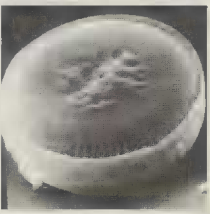

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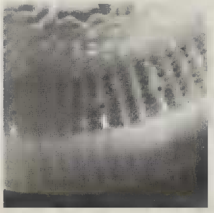

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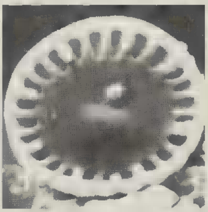

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The other rich material originates from France, in the Tours-Orléans section of the Loire. Examinations showed *C. caspia* to occur there in fairly large number in summertime (about 10 to 100 thousand ind./l). This species was also found in the autumn sample taken from the Toulouse section of the Canal du Midi. On a few occasions, its presence was recorded in the Hamburg section of the Elbe, and the section of the Vistula river below the Goczalkowice Reservoir. We have some data from a small lake near Karolinka (in the mountain West Beskidy).

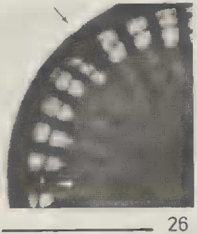
DISCUSSION

During the morphological investigation of *C. caspia* the greatest difficulty was encountered in connection with the light microscopic identification of this species. It is seldom possible to observe each morphological feature, that is necessary for proper identification. In the case of specimens less than 8-10 μm it is hardly possible to count the exact number of costae (which in addition vary within a wide range). Moreover, the marginal strutted processes are then not visible. For the Danube material, the pattern of protuberances in the central part of the valve face, appearing as fairly conspicuously spotted under the light microscope, was the most useful feature for identification (Fig. 5-17). In the samples from the Loire, the tangential depression in the central part of the valve face, and the definite inner margins of alveoli could be seen quite well (Fig. 2-4).

Since *C. caspia* occurs together with the more frequent *C. atomus* Hust., it is often difficult to differentiate the two, particularly in the case of small specimens. The costae of *C. atomus* are spaced at greater distances and are not of equal length; the marginal strutted processes located at every 3-4 stria are generally well noticeable as minute spots. The central siliceous field of *C. atomus* is not spotted with minute protrusions and there is no tangential depression. This difference was also pointed out by Nagumo & Kobayasi (1985).

We can presume on the base of Genkal & Makarova's paper (1986), that under the light microscope *C. caspia* cannot be distinguished, or is difficult to

Figs. 20-25 : SEM. Specimens from the Danube. Scale : 2 μm. Figs. 20-21 : External view of valve face with the characteristic feature of central part, and with pore of central strutted process and labiate process (arrowed). Figs. 22 : External view with costae dichotomously branched and with the pore of central strutted process (arrowed). Fig. 23 : External view showing the pores of marginal strutted processes and the tangential fold of central part. Fig. 24 : External view of a detail of valve mantle, with the pores of marginal strutted processes (arrowed), showing the costae and the external feature of alveoli. Fig. 25 : Internal view of valve face showing the marginal strutted processes on the costae, with two satellite pores, the central strutted process in excentric position with three satellite pores, and the tangential fold of central part.



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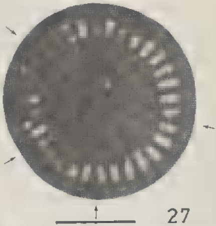

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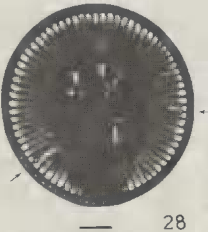

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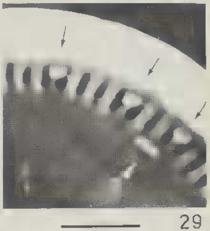

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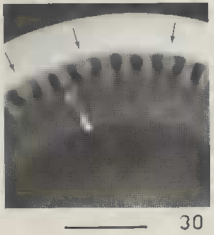

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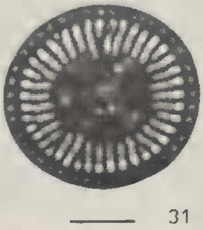

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separate from the *C. affinis* (Proshkina-Lavrenko et Makarova) Makarova et Genkal thus labelled by Proshkina-Lavrenko (1959, 1963) or Genkal & Makarova (1986). This latter species is, however, known as a marine one, and we have no information concerning its freshwater occurrence.

Our experiments showed that in a particular water body, *C. caspia* can generally be detected on the basis of EM studies if it is fairly abundant.

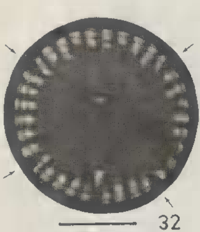
The EM studies clearly suggest that the morphological features of *C. caspia* depend on the degree of silicification of the frustules. Consequently, it is also likely that the populations of this species inhabiting various waters differ with regard of some features.

Comparison with literature data shows a high morphological variation for this species concerning both costae and alveoli. Our minimum and maximum values for the number of costae in 10 µm correspond with the literature but the average values are lower. However, only the data by Hasle (1962), and Genkal & Makarova (1986) were used for comparison purposes because the other papers did not contain average values. Nevertheless, our lower values might be the result of the fact that the number of costae in 10 µm was originally established by using Genkal's method (1977); i. e. we calculated the number of costae in 10 µm from the total number of costae of the circumference. Values thus obtained by Genkal & Makarova (1986) were also lower than those they measured according to Anonymous (1975). Similarly, if the number of costae of specimens in the two TEM micrographs shown in Archibald's paper (1983) are estimated after Genkal's method (1977), the values obtained (15,7 respectively 16,3 in 10µm) will be those of the lower limit again (in Archibald's paper the number of striae is 16-28 in 10 µm).

These facts indicate, that there are problems with the measuring methods of the small-sized species of Thalassiosiraceae. We suggest that Genkal's method (1977) should be followed, and think it would be appropriate to revise the international recommendation (Anonymous, 1975; Ross *et al.* 1979).

As regards the morphology of costae, two essentially different types can be distinguished, with several transitions between them.

Figs. 26-28, 31 TEM, 29-30 SEM. Scale : 2 µm – Fig. 26 : Detail of valve margin. Structure of alveoli with little pores circular, concentric ring run parallel with the mantle, the marginal strutted process and the pore of labiate process (arrowed, specimen from the Danube). Fig. 27 : Valve with four marginal strutted processes in asymmetrical position (arrowed, specimen from the lake Saisser). Fig. 28 : Valve with three central strutted processes and two labiate processes (arrowed, specimen from the periphyton of the Danube). Figs. 29-30 : Detail of internal part of valve mantle showing the costae, the marginal strutted processes (arrowed with black), and the different position of labiate process (arrowed with white, specimens from the Danube). Fig. 31 : Characteristics features of a slightly silicified valve with the long costae, with the «external» (white) and «internal» (grey) part of alveoli. The pore of the labiate process is arrowed (specimen from the Danube).



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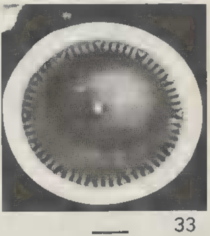

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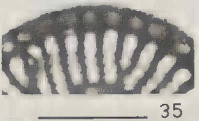

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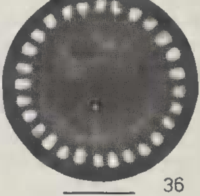

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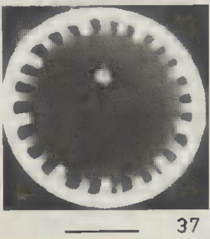

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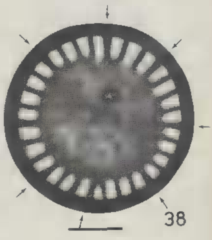

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In one case, the costae are short, radially straight (Figs. 25, 26, 32, 37, 44) and arched following the course of the valve mantle (Fig. 39). The costae in the figures presented by Genkal & Makarova (1986) and the TEM micrograph 31a and the carbon replica photos 11,30 from Hasle's paper (1962) are more or less identical to those. In the other case, the costae are long, radial, more or less straight, some of them dichotomically branching. This type can be well observed in TEM micrographs (Figs. 28, 31, 40) as well as the SEM pictures showing the superficial morphology of the valve (Figs. 20, 23, 42). In SEM micrographs, showing the inner part of the valve mantle, the costae appear very similar to those of the first type because of their extensions which are undistinctly seen in the photographed specimens (Figs. 29-30, 33).

Transitions were also found between the two types above (Fig. 27. 42-44). Similar transitional forms can be seen in the micrographs presented in figures 513, 514 by Archibald (1983), in figures 5, 6, 10, 12-15, 29 by Hasle (1962), and figures 15-19 by Nagumo & Kobayasi (1985). In the latter ones, (pictures 17, 19 : SEM, inner part of the valve face) the longer costae are visible.

All these examinations confirm that the broad scale of transitions between the short and long forms of costae depends on the degree of silicification of the frustule. Valves less silicified have definite long costae on the valve face. On the inner part of the valve face only the arched short part of each costa is discernible. With the progression of the silicification the costae are less distinct over all their length, and only their short arched marginal parts are visible.

The length of costae and the ratio between the diameter of the valve face with costae and the diameter of the central part of valve face having no regular structure has been given in each paper published so far, so was the ratio characterizing a particular species (generally 0.2-0.3). Our data clearly show that this ratio is of no particular importance, and should not be used in identification. The value of (D-Dc)/D (the diameter of the valve minus the diameter of the central part of the face divided by the diameter of the valve) was generally found to be 0.3 in the case of specimens with short costae. With specimens

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Figs. 32, 34-36, 38 TEM, 33, 37 SEM. Scale : 2 µm. — Fig. 32 : TEM structure of a valve showing the special feature of alveoli with little round pores and concentric rings, with four marginal strutted processes in regular position and the pore of labiate process (arrowed, specimen from the Danube). Fig. 33 : Internal view of valve, showing the last pleura often forms a thickened ring (specimen from the Danube). Fig. 34 : Structure of the valvocopula (specimen from the Danube). Fig. 35 : Detail of valve margin showing the long costae and the spine-like structure at the rim of mantle (specimen from the Danube). Fig. 36 : TEM structure of a valve more heavily silicified, with short costae and rounded alveoli (specimen from the Tisza). Fig. 37 : Internal view of a valve more heavily silicified showing the short costae, rounded alvoeli and the processes (specimen from the Tisza). Fig. 38 : TEM structure of a valve more heavily silicified showing the feature of alveoli. Among thin, longitudinal timber-like structures there are mainly long-shaped irregular pores (marginal strutted processes arrowed, specimen from the Loire).

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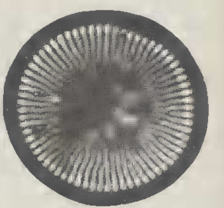

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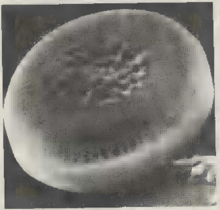

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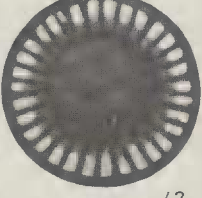

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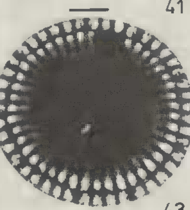

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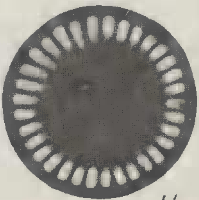

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less silicified and having long costae this value was often more than 0.5 if the measurements were made on the basis of TEM micrographs or SEM pictures showing the valve face in external view. When the measurements were performed on SEM micrographs or SEM micrographs taken on the inner part of the valve face, and when parts of costae near the central part could not be clearly seen, the value for (D-Dc)/D in the case of the same specimen was only 0.2-0.3 again. As a matter of fact, the shape and structure of alveoli also depends on the degree of silicification, i.e. the shape and length of the costae.

SEM micrographs show that the more or less elongated oval chambers of the alveoli on the inner part of the valve face are identical in types with either short or long costae. TEM micrographs and SEM pictures of the valve face, however, point to a conspicuous difference between the afore-mentioned two types. The alveoli of specimens with short costae and heavier silicification are definitely rounded, closed towards the centre and the valve mantle (Figs. 25, 36, 38). Specimens silicified in a lesser degree and having long costae usually exhibit a definite alveolar margin towards the valve mantle, while towards the centre the alveolar margin is only indefinitely closed, and the perforated (areolated) outer layer runs a long distance radially (Figs. 20-23, 28, 31, 40).

In the case of transitions (which are frequent in the material from the Loire) the chambers of the alveoli are less definitely closed towards the valve mantle or towards the centre, or either (Figs. 42, 44). The TEM micrographs presented by Archibald (1983) and Nagumo & Kobayasi (1985) are similar to those. Sometimes the perforated external walls of the alveoli are not made up of longitudinal perforations, but of circular ones; concentric rings can be seen in them (Fig. 32).

On the basis of EM studies reported here, the morphological features necessary for good identification of *C. caspia* can be summarized as follows :

– The cells are rectangular in girdle view.

– The height of frustule is greater than its diameter.

– The most decisive features are the presence of the central strutted process and the light undulation of the central part of the valve face, i.e. the fact that on the inner part of valve face the alveoli are distinctly rounded both toward valve mantle and the centre. The former feature is usually well observable also in TEM micrographs, in the SEM micrographs, however, it is indisputably visible.

Figs. 39, 41 SEM, 40, 42-44 TEM. Scale : 2 µm. — Fig. 39 : Internal view showing the form of costae, alveoli and tangential fold of the central part (specimen from the Danube). Fig. 40 : TEM structure of a slightly silicified valve showing the feature of alveoli, the costae frequently branched and the pore of the labiate process at the middle of a costa (arrowed, specimen from the Danube). Fig. 41 : External view of a frustule slightly silicified showing the valve mantle, the long costae and the characteristic feature of central part (specimen from the Danube). Figs. 42-44 : TEM structure of the valve face. The structure of alveoli form a transition from the slightly to the more heavily silicified valves (specimens of Figs. 42-44 from the Loire, Fig. 43 from the Danube).

The light undulation can only be definitively recognized in SEM pictures. The other morphological features (the number of costae and strutted processes in 10 µm, the shape, length of costae, alveoli, the ratio of the central part of valve diameter) can vary within a large range and are thus of little value with regard to identification.

In these studies *C. caspia* was recorded about 100 times from [redacted] few European rivers and lakes. The studied waters are generally located very far from another geographically; they do not belong to a common, large catchment basin. *C. caspia* was found most frequently in the Danube and its tributaries, and in the Loire but in these rivers its frequency is not necessarily the highest. Probably only the sampling distribution and prospection activity are in this way reflected. Consequently the sporadic occurrence of *C. caspia* often very scarce, is highly probable everywhere in Europe and moreover in worldwide freshwaters (Archibald, 1983; possibly Giffen, 1963; Nagumo & Kobayasi, 1985). The same species was recorded by Genkal & Kuzmin (1979b) from a few rivers and lakes in the European part of the Soviet Union, although they identified it as *C. atomus* (see Pl. 1, Fig. 6, Pl. 3, Fig. 4).

There is no doubt that *C. caspia* was described from a sea with fairly low salinity and has been regarded by Proshkina-Lavrenko (1959) as a euryhaline and eurythermous species. Literature data and our own investigations have unequivocally shown that this species occurs in sea water, brackish water as well as freshwaters during the whole year. On the basis of our studies it has not been possible yet to point our morphological differences between the salt water and freshwater populations of this species.

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